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Reviews and invited commentary

Understanding the causes, prevention and treatment of osteoporosis (part 1): the structure of bone and the remodelling proces

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KEY WORDS

bone architecture
bone remodelling cycle
osteoblasts
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osteocytes
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ABSTRACT

Osseous tissue, or bone, is a living tissue, composed of a mixture of organic and non-organic substances. Bone tissue can be described according to its level of structure: macro-, micro-, sub-micro-, nano-, and sub-nano-. At the macroscopic level, bone can be classified as cortical or cancellous, organised microscopically as osteons and trabeculae respectively. At the sub-microstructure level bone can be lamellar or non-lamellar, while at the cellular level, bone consists principally of three main cell types: osteoclasts, osteoblasts, and osteocytes, which interact to continuously regenerate the bone matrix. Bone is continuously modelling/remodelling itself throughout an individual's life, allowing the skeleton to increase in size during growth, respond to physical stresses, and repair structural damage due to fatigue, failure or trauma. Bone homeostasis is only maintained if the processes of resorption and formation are closely coupled. Understanding the micro- and macro-architecture of bone and the processes involved in bone remodelling are crucial in the diagnosis of osteoporosis and the evaluation of the effectiveness of treatments and interventions. The purpose of this article (the first of a three-part feature) is, therefore, to describe the structure and function of bone, the cells involved in bone remodelling, and the process of remodelling itself.

The framework of the human body is provided by the 206 separate bones of the skeleton. Bones provide the structural framework of the body, which supports soft tissues and the surface of which act as points of attachment for tendons and the majority of skeletal muscles. The bones also protect many internal organs, including cranial bones to shield the brain, vertebrae to protect the spinal cord, and the rib cage, which acts as a defensive barrier for the heart and lungs. As well as the bones acting as a protective shield for vital organs, it also acts as a storage facility for a number of minerals, primarily calcium (99% of all body calcium is stored within bone) and phosphorus, which contribute to the overall strength of bone. Bone has the ability to release these minerals into the bloodstream to ensure that critical mineral balances are maintained and to allow the distribution of minerals to other organs. Red bone marrow, which produces red blood cells, white blood cells, and platelets by a process termed hemopoisis, is also contained within certain parts of bones. The skeleton itself can be subdivided into the axial (80 bones) and appendicular (126 bones) components; the axial skeleton forming the axis of the body, supporting and protecting the organs of the head, neck, trunk and spine, while the appendicular skeleton (from the Latin appendic meaning to hang-to) comprises the bones of the upper and lower extremities and the bony girdles that anchor the appendages to the axial skeleton. The bones themselves can be further classified according to their size, shape, function, and the proportion of compact and cancellous bone tissue: long; short; flat; irregular; sesamoid; and sutural.

Organisation of bone

Bone, or osseous tissue, is an extremely complex, organised and specialised connective tissue. It is highly heterogeneous, partially because of its adaptation to resist different, complex and varying stresses (Dudley and Spiro, 1961). Bone is a relatively hard and lightweight composite material, which is strong, but relatively elastic (Nordin and Frankel, 2001). Bone has relatively high compressive strength, of about 170 MPa (1800 kgf/cm^2) (Schmidt-Nielsen, 1984), comparatively poor tensile strength of 104–121 MPa, and very low shear stress strength of 51.6 MPa (Turner et al., 2001). This means it resists pushing forces well, but not pulling or torsional forces. While bone is essentially brittle, it does have a significant degree of elasticity, contributed chiefly by collagen. All bones consist of living and dead cells embedded in the mineralized organic matrix that makes up the osseous tissue. The mechanical behaviour of bone, essentially its behaviour due to forces and moments, according to Frankel and Nordin (2001) is affected by its mechanical properties, its geometric characteristics, the loading mode applied, the direction of the loading, and both the rate and frequency of this loading. Bone exhibits many

orders of structures: the macrostructure (cancellous and cortical bone), the microstructure (haversian systems, osteons, single trabeculae - 10 to 500 µm), the sub-microstructure (lamellae - 1 to10 µm), the nanostructure (fibrillar collagen and embedded mineral - ~300 hundred nm to 1 µm), and the sub-nanostructure (mineral, collagen, and non-collagenous organic proteins - <300 nm). Macroscopically, there are two types of osseous tissue (Figure 1): cortical bone (compact), which constitute approximately 80% of the skeleton and are predominantly found in the shafts of the long bones such as the femur and tibia; and cancellous bone (trabecular or spongy), mainly found at the end of long bones and inner parts of flat bones, like the sternum, and irregular bones, such as the vertebrae. Essentially, the tissues are biologically identical; they simply differ in how the microstructure is arranged. The relative proportions of the type of bone vary dramatically depending on the skeletal site; for example the cortical: trabecular ratio at the vertebra is approximately 25:75, while at the head of the femur it is 50:50 (Dempster, 2006). While the diaphysis of a long bone is comprised of layers of cortical bone, the metaphysis and epiphysis are both constructed of cancellous bone, inside a thin layer of cortical bone (Figure 2). The hard outer layer of bones is composed of compact bone tissue, so-called due to its minimal gaps and spaces. Cortical bone is much denser than cancellous bone with a porosity ranging between 5% and 30% (Hall, 2007). In the cortical bone, the periosteum is the outer fibrous structure containing blood vessels, nerve ending and the bone cells. The periosteum is anchored to the bone with Sharpey's fibres, which penetrate into the bone tissue. The endosteum is a membranous sheath, which inhabits the inner surface of cortical bone and is in direct contact with marrow (Brandi, 2009).

The basic first level structure, or microstructure, of cortical bone is the osteon or Haversian system (Figure 3a). The osteons are cylinders consisting of four main parts: lamellae, lacunae, canaliculi, and a central canal. The lamellae are essentially rings of matrix, which contain calcium and phosphates, while the lacunae are small cavities housing osteocytes. Canaliculi are spokes that radiate from the lacunae, while the central canal contains blood vessels, nerves and lymphatic vessels (Figure 3b). Cancellous bone does not consist of osteons; the basic first level structure (microstructure) of trabecular bone being trabeculae. It resembles a lattice of thin struts that are constructed of columns of bone (Figure 4) containing osteocytes, lacunae, canaliculi and lamellae. Lighter and less dense than cortical bone, cancellous bone accounts for the remaining 20% of total bone mass but has nearly ten times the surface area of compact bone, with a porosity range of 30–90% (Hall, 2007). Bone marrow can be found in almost any bone that holds cancellous tissue. In newborns, all such bones are filled exclusively with red marrow, but as the child ages it is mostly replaced by yellow, or fatty marrow. In adults, red marrow is mostly found in the marrow bones of the femur, the ribs, the vertebrae and pelvic bones. If, for any reason, there is an alteration in the strain that cancellous bone is subjected to, there is a rearrangement of the trabeculae. So the microscopic difference between compact and cancellous bone is that compact bone consists of Haversian sites and osteons, while cancellous bones do not. Also, bone surrounds blood in compact tissue, while blood surrounds bone in cancellous tissue. In addition to differing in appearance, the two types of bone also differ dramatically in their mechanical properties, particularly in the qualities of strength and elongation. Interestingly the compressive strength of cor-

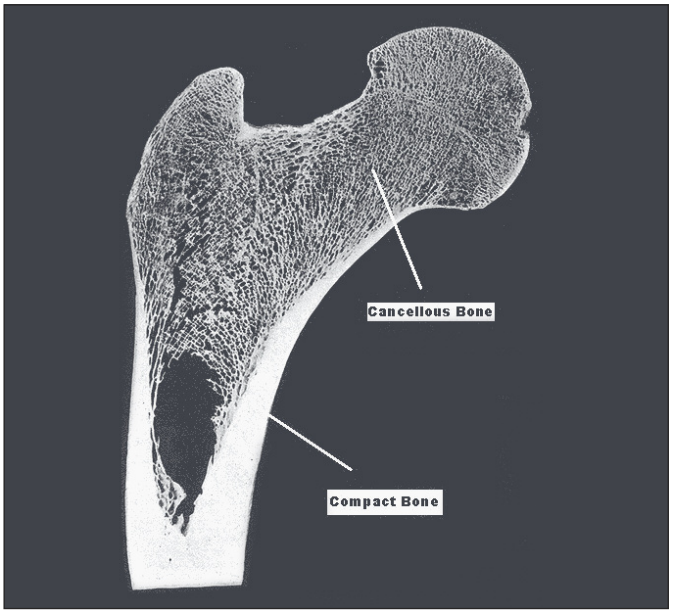

Figure 1. Femur head comprising both compact and cancellous bone (adapted from Henry Gray's *"Gray's Anatomy"*; Lithograph 247 femur)

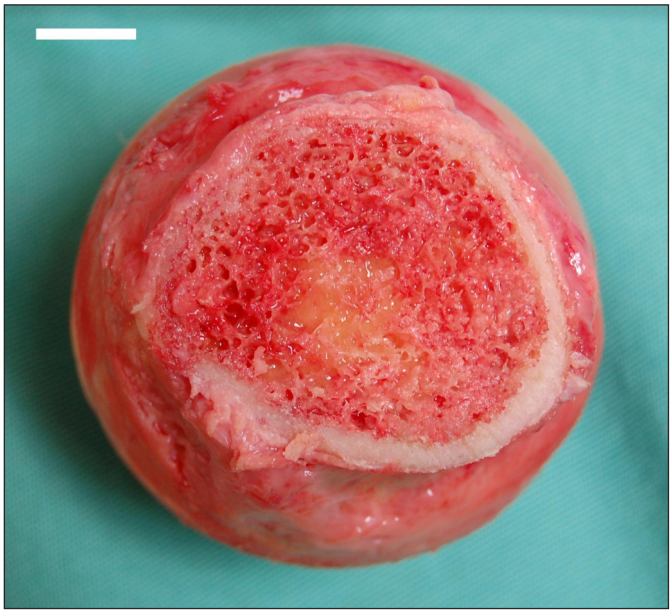

Figure 2. Femur head cut-away to reveal compact and cancellous bone, red and yellow marrow: white bar represents 0.01m (used with permission from Steven Fruitsmaak)

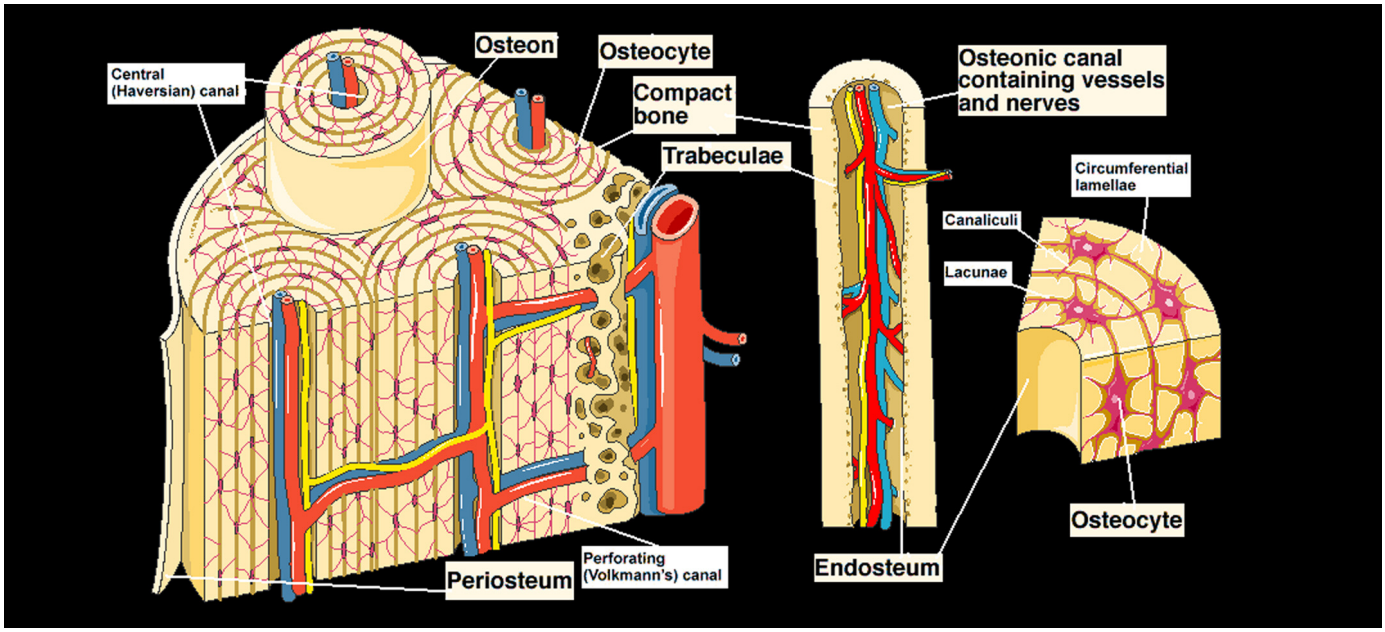

Figure 3a. Bone structure (after Hawkey, 2008; artwork originally adapted and used with permission from Les Laboratoires Servier)

tical bone is, according to Hamill and Knutzen (2009), greater than that of concrete, wood or glass. This can be demonstrated by bone's ability to tolerate large impact loads and resist bending: those experienced during the step and jump phases of the triple jump for example; reported to be as high as 17 times an individual's body weight (BW) in the vertical axis (Hawkey and Scattergood, 2007). While cancellous (trabecular) bone does not have the strength of cortical bone, it is reported to be able to withstand more deformation before failure (Hamill and Knutzen, 2009).

At the sub-microstructure level, bone consists of two main types of tissue, which can be identified microscopically according to the pattern of collagen forming the osteoid (collagenous support tissue of type I collagen embedded in glycosaminoglycan gel): primary (non-lamellar) bone; and secondary (lamellar) bone. Non-lamellar bone, also known as coarse fibred, woven or immature bone is characterised by the presence of randomly oriented coarse collagen fibres (Figure 5). It is mechanically weak and is produced when osteoblasts produce osteoid rapidly, which occurs initially in all fetal bones (later replaced by more resilient lamellar bone). In adults woven bone is created after fractures or in Paget's disease. Lamellar bone tissue, often referred to as mature bone, is identifiable by the presence of collagen fibres arranged in parallel layers or sheets, called lamellae (Figure 6). Lamellar bone, which has a regular parallel alignment of collagen into sheets (lamellae), is mechanically strong. Lamellar bone, which makes its first appearance in the fetus during the third trimester, is mechanically stronger than woven bone

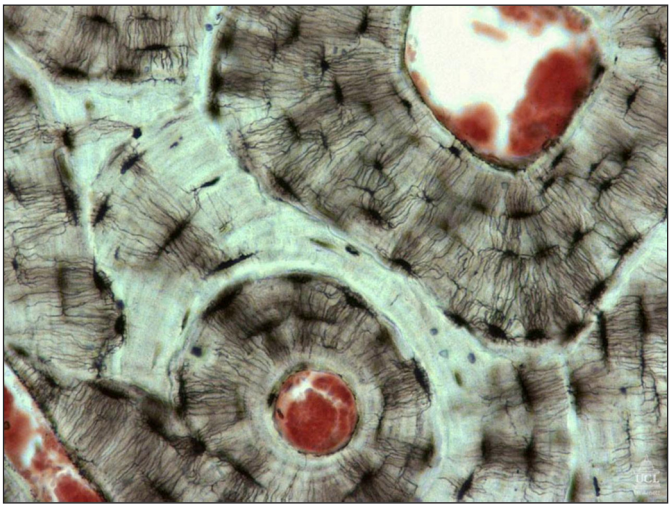

Figure 3b. Compact bone – blood vessels (red) and osteocytes with caneculli (black) clearly visible (used with permission from Tim Arnett/Bone Research Society)

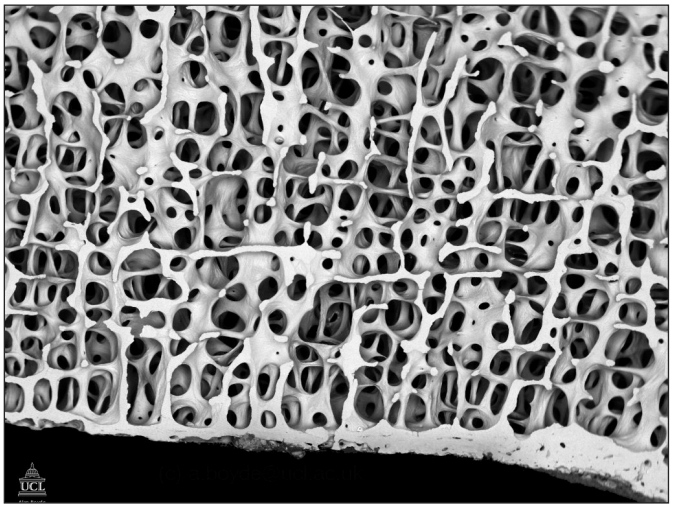

Figure 4. Cancellous bone. Trabecular structure clearly visible (used with permission from Alan Boyde/Bone Research Society)

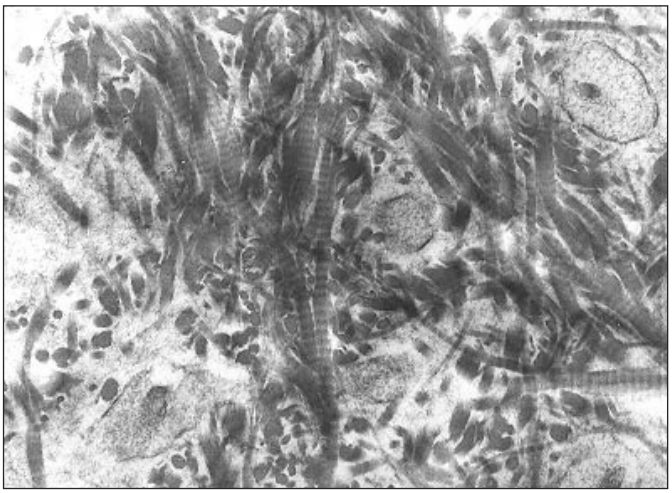

Figure 5. Woven (non-lamellar) bone (used with permission from Robert M. Hunt)

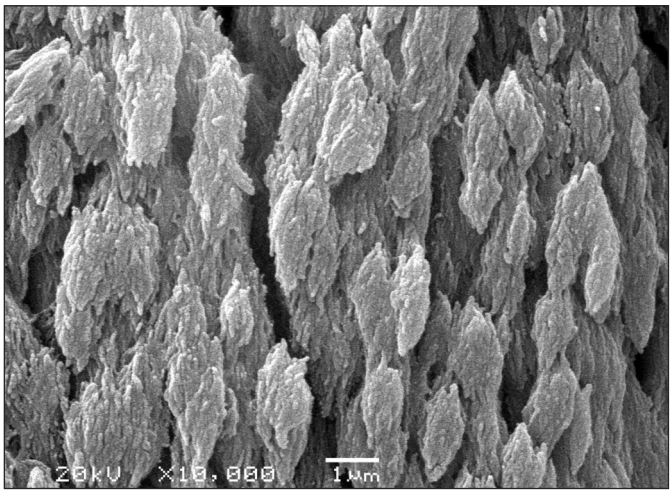

Figure 6. Lamellar bone (used with permission from S. Bertazzo)

and is filled with many collagen fibers parallel to other fibers in the same layer (these parallel columns are called osteons). In cross-section, the fibres run in opposite directions in alternating layers, much like in plywood, assisting in the bone's ability to resist torsion forces. After a fracture, woven bone forms initially and is gradually replaced by lamellar bone during a process known as bony substitution. Compared to woven bone, lamellar bone formation takes place more slowly. The orderly deposition of collagen fibers restricts the formation of osteoid to about 1 to 2 μm per day (Salentijn, 2007).

At the nano- and sub-nanostructure level, bone can be referred to as a two-phase (biphasic) composite material: one phase being mineral (non-organic); the other comprised of collagen and ground substances, known as the organic matter (Figure 7). The organic matter consists of type I collagen fibres embedded in proteoglycans and glycoproteins (Bertazzo and Bertran, 2006; Bertazzo et al., 2006). Collagen molecules secreted by osteoblasts self-assemble into fibrils with a specific tertiary structure having a 67 nm periodicity and 40 nm gaps between the ends of the molecules. The collagen fibres (essentially bundles of fibrils) act as a soft hydrogel template and resist pulling forces. The in-organic matter is made up of stiffening substances to resist bending and compression; the bone mineral is an analogue of crystals of calcium phosphate and hydroxyapatite (HA)Ca10(PO4)6(OH2). Nanocrystalline HA which is typically 20-80nm long and 2-5nm thick comprises 70% of the bone matrix (Bertazzo and Bertran, 2006; Legros et al., 1987; Field et al., 1974). It is this association of hydroxyapatite with collagen fibres which is responsible for the hardness of bone. Bone consists of a dense, layered, regular network of collagen fibres embedded in a hard, solid ground substance (Watkins, 2010). The ground substance is generally referred to as bone salt and consists of a combination of calcium phosphate, calcium carbonate, magnesium, sodium, and chlorine. Non-collagenous organic proteins, including phosphoproteins, such as osteopontin, sialoprotein, osteonectin, and osteocalcin, may function to regulate the size, orientation, and crystal habit of the mineral deposits (Rho et al., 1998). In materials such as bone (and non-biological examples such as fibreglass), where a strong brittle material is embedded in a weaker, but more flexible material, the substances combined are stronger, in relation to their weight, than either substance alone (Bassett, 1965). A number of factors, such as site, age, dietary history and disease have an influence on bone composition (Kaplan et al., 1994). The mineral portion of bone is comprised mainly of calcium and phosphate, giving bone its solid consistency, which accounts for 60-70% of its dry mass. Water is relatively abundant in living bone and accounts for approximately 25% of its total mass: 85% of which is contained in the organic matrix, collagen fibres, ground substance, and hydration shells surrounding bone crystals; the remaining 15% being housed in the canals and cavities

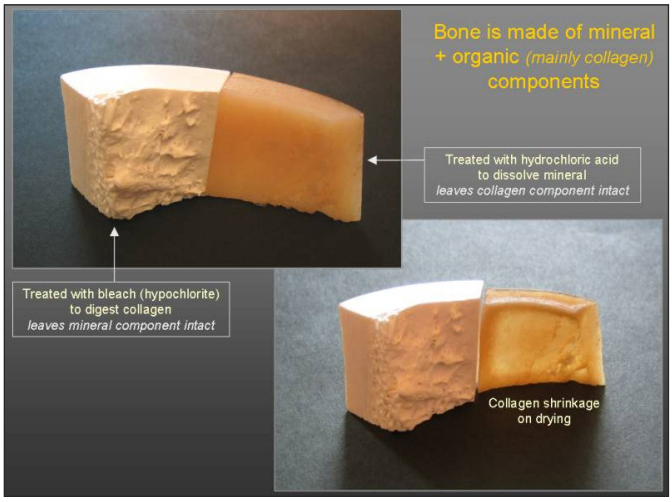

Figure 7. Biphasic bone: mineral and collagen portions (used with permission from Tim Arnett/Bone Research Society)

transporting nutrients to the bone tissue (Frankel and Nordin, 2001).

Bone modelling and remodelling

Bone has the ability to adapt its size and shape in response to mechanical loads (Brandi, 2009). This ability can be described through two similar, but distinct, processes: bone modelling and bone remodelling. The bone modelling process occurs not only during the growth period, but also during adulthood. It can be viewed as the bone directly responding to mechanical loading by initiating bone formation, without prior bone resorption. This process can be seen in tennis players, where the radius of the playing arm shows larger external diameter and a thicker cortex than the non-playing arm (Brandi, 2009). The converse is evident in those who are confined to long-term bed-rest, or astronauts in spaceflight, where significant bone loss is experienced (Hawkey, 2003a; 2003b). The process of bone remodelling is a surface-based phenomenon involving the removal of bone matrix (resorption) and the deposition of new bone (inversion). There are three principal cells that are active in this process within the bone matrix: osteoclasts, osteoblasts, and osteocytes. Osteoclasts are large multinucleate cells, which resorb bone and remove degraded material (Figure 8a). They are located on bone surfaces in what are called Howship's lacunae or resorption pits. These resorption pits are left behind after the breakdown of the bone surface. Osteoclasts are derived from a monocyte stem-cell lineage, and are equipped with phagocytic-like mechanisms similar to circulating macrophages. Osteoclasts mature and/or migrate to discrete bone surfaces. Upon arrival, active enzymes, such as tartrate resistant acid phosphatase, are secreted against the mineral substrate (Figure 8b). This creates an acidic environment on the surface of bone, which dissolves the bone's mineral content. Once the mineral content of the bone has been dissolved, enzymes released from osteoclasts remove the remaining collagenous bone matrix to complete the process of resorption. The rate at which osteoclasts resorb bone is inhibited by calcitonin and osteoprotegerin. Calcitonin is produced by parafollicular cells in the thyroid gland, and can bind to receptors on osteoclasts to directly inhibit osteoclast activity. Osteoprotegerin is secreted by osteoblasts and is able to bind RANK-L, inhibiting osteoclast stimulation (Boulpaep et al., 2005). Following resorption, osteoblasts (Figure 9) are then attracted to the resorption cavity and start to produce and deposit organic matrix called osteoid, primarily composed of Type I collagen. Minerals start to crystalise around the collagen scaffold to form hydroxiapetite, the primary in-organic constituent of bone, which contains calcium phosphate. The product of this process is new bone matrix being deposited and mineralised (Helfich, 2003; Phan et al., 2004; Hawkey, 2007). As osteoblasts form new bone tissue many of them become embedded into the matrix they create; causing them to differentiate into osteocytes (Figure 10), while other, non-active osteoblasts called bone lining cells, cover all of the available bone surface and function as a barrier for certain ions. Osteocytes are, therefore, essentially mature osteoblasts trapped within calcified bone, and are believed to transmit information about mechanical forces in response to deformations of bone caused by muscular activity; which then directs bone remodelling to accommodate these forces (You et al., 2000; Ehrlich and Lanyon, 2002). Bone remodelling differs from modelling in that it is initiated by a period of resorption lasting approximately two weeks, while osteoclasts erode an area of bone. The duration of this remodelling process is approximately three to six months; the majority of this period being taken up with bone formation (Manolagas, 2000). This remodelling process (often termed the bone remodelling cycle) can be clearly identified into four distinct phases: quiescence/activation; resorption, reversal, and formation (Figure 11). It has been estimated that this bone remodelling process, which is essentially the interaction of osteoclasts and osteoblasts, is in action, at any one time, in approximately 500,000 sites throughout the body, which equates to approximately 10% of the bone surfaces in the adult skeleton (Marx, 2004).

The process of bone responding to changing loads is known as Wolff's Law, which describes the remodelling of bone and is influenced and modulated by mechanical stresses (Buckwater et al., 1995). Specifically, Wolff's law states that "every change in the form and function of a bone or of their function alone is followed by certain definitive changes in their internal architecture and equally definite secondary alteration in their external conformation, in accordance with mathematical laws" (Keller and Spengler, 1989). Repeated stress, such as weight-bearing exercise or bone healing, results in the bone thickening at the points of maximum stress (Wolff's law). It has been hypothesized that this is a result of bone's piezoelectric properties, which cause bone to generate small electrical potentials under stress. (Netter, 1987). All of the bone in an adult's skeleton is replaced every ten years (Marx, 2004), with approximately 10% renewed by remodelling every year (Manolagas, 2000). Due to this remodelling process, bone mass is continually changing throughout life, during skeletal growth, consolidation and involution. In healthy young adults, total bone mass remains relatively constant, indicating equal rates of bone depositing and resorption. However, the remodelling process is not uniform, with some bones, or areas of bone, experiencing very different levels of remodelling. A good example of this is the distal portion of the femur, which is fully replaced approximately every six months; the shaft of the femur, however, is remodelled much more slowly. Although the exact processes are not yet known, it is believed that the interaction between osteoclasts and osteoblasts changes throughout life. During the younger years bone goes through a growth period, dur-

ing which approximately 90% of peak bone mass is deposited. During this period the osteoblast cells are more prevalent than osteoclast cells, meaning more bone is laid down. A consolidation period of approximately 15 years then follows, which increases bone mass further until peak bone mass is reached in the mid-twenties. Involution, a stage where bone resorption begins to supersede bone deposition, then typically starts between the ages of 35 and 40 with cortical and trabecular bone being lost with advancing age in both sexes according to bone type and anatomical site. Generally, women lose 35-50% of trabecular and 25-30% of cortical bone, while men lose 15-45% and 5-10% of trabecular and cortical bone respectively, over their lifetime (Riggs and Melton, 1986). Bone loss starts initially on the surfaces of bone, so any alterations in bone mass are seen earlier, and to a greater extent in areas with a higher percentage of trabecular bone as opposed to those consisting principally of cortical bone (Brandi, 2009); the head of the femur and the vertebrae for example. Bone homeostasis is only maintained if the opposing (or complimentary) actions of osteoclasts and osteoblasts are balanced, meaning resorption and formation are closely coupled. A defect in either process can result in increased bone accumulation (osteopetrosis), or in increased bone turnover (osteoporosis). In the second part of this feature, the mechanisms and risk factors of osteoporosis will be discussed as will the efficacy of various techniques employed to quantify bone health.

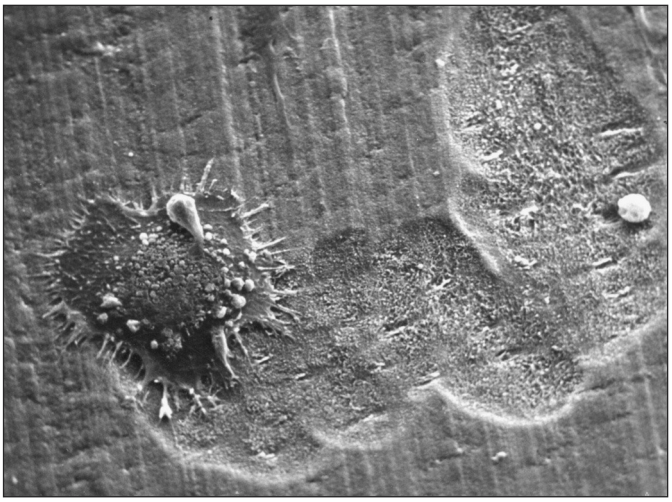

Figure 8a. Osteoclast Cell (used with permission from Alan Boyde/Bone Research Society)

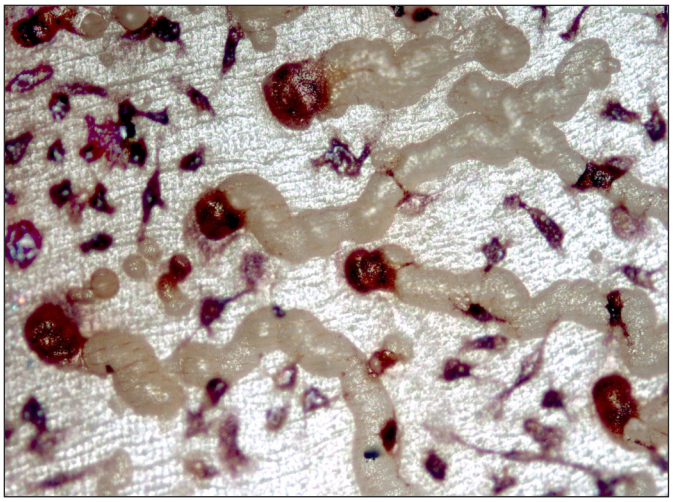

Figure 8b. Osteoclast Cells resorbing bone (used with permission from Tim Arnett/Bone Research Society)

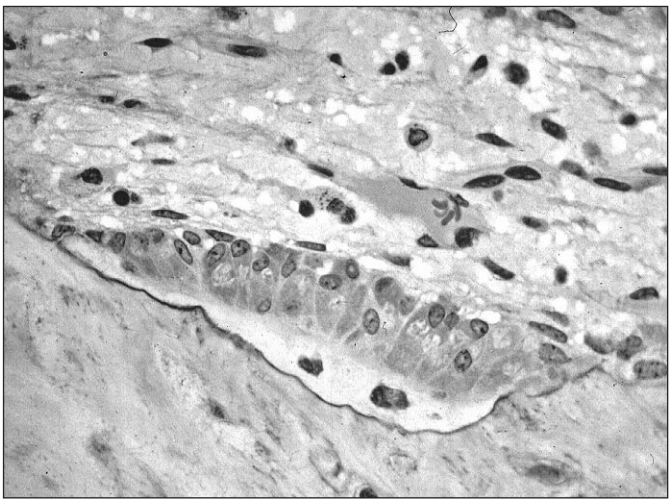

Figure 9. Osteoblast Cell (used with permission from Robert M. Hunt)

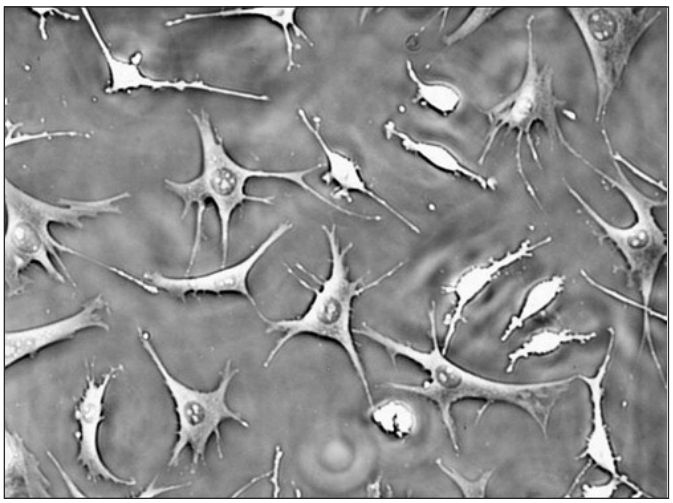

Figure 10. Osteocyte Cells (used with permission from the Southwest Research Institute funded by NIH grant AR046798)

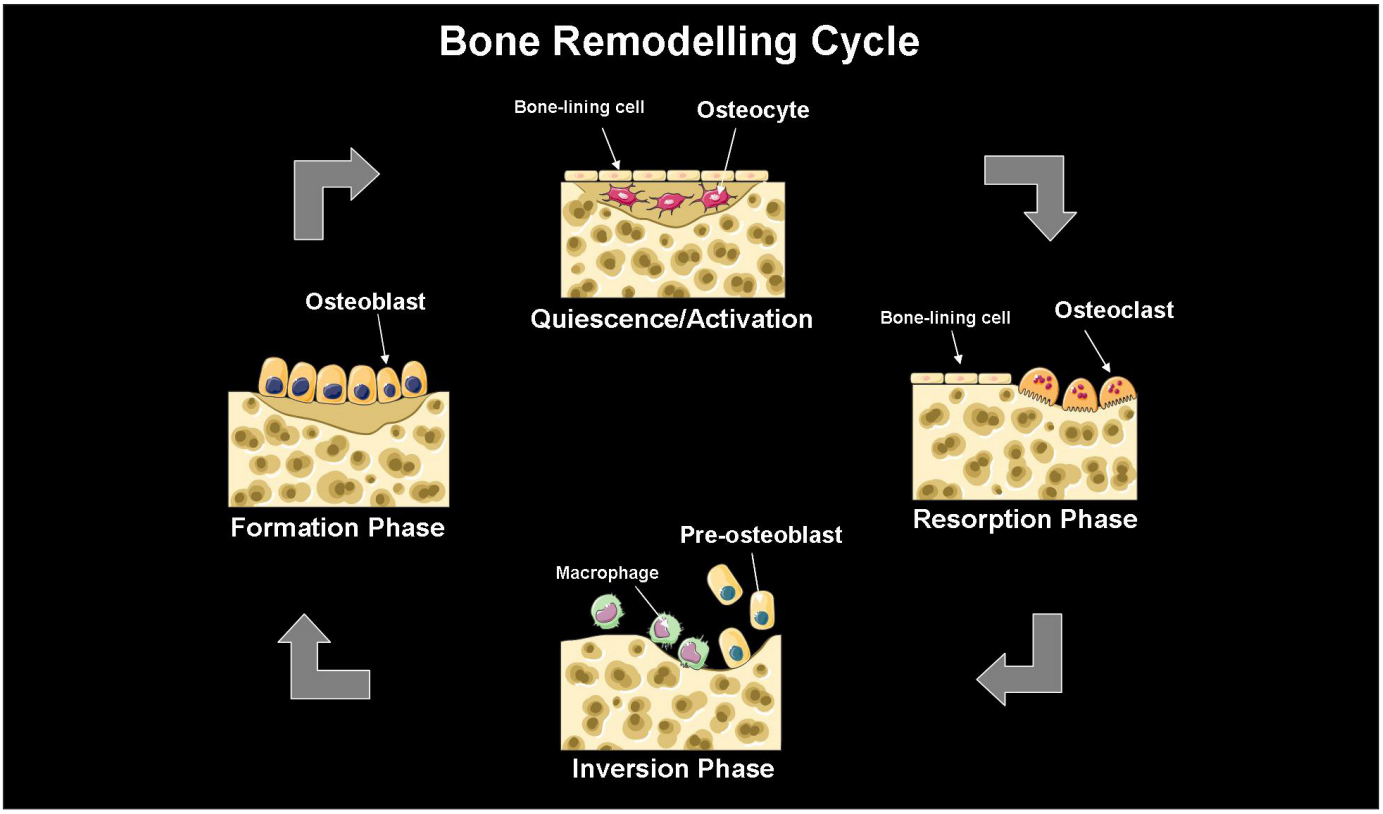

Figure 11. Simulated representation of the Bone Remodelling Cycle (after Hawkey, 2008; artwork originally adapted and used with permission from Les Laboratoires Servier)

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